AN IODOMETRIC METHOD FOR THE DETERMINATION OF SODIUM IN SMALL AMOUNTS OF SERUM.*

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Several years ago one of us described a gravimetric method for the determination of small amounts of sodium in ashed serum (1). In this method the sodium is precipitated as the pyroantimonate

$$K_2H_2Sb_2O_7 + 2NaCl \rightleftarrows Na_2H_2Sb_2O_7 + 2KCl$$

and the precipitate dried and weighed. Subsequently Kramer and Tisdall (2) found that the proteins of the serum do not interfere with the precipitation so that it is possible to eliminate the ashing. In this and in several other laboratories satisfactory results have been obtained with these methods. However, other investigators have not been so fortunate. We have always felt that the source of error must lie for the most part in the filtration. The precipitate is finely crystalline and is retained only by very dense filters. It is quite soluble in water and not altogether insoluble even in 30 per cent alcohol. The drying of the precipitate is carried out at 120° C. and the length of time required for the best results seems to vary with the amount of precipitate that has already accumulated on the filter. When the ash of serum is used it must be made alkaline before adding the precipitating reagent. Substances that are insoluble in alkaline solutions are precipitated and weighed with the sodium pyroantimonate. With serum or plasma the error thereby introduced is small, but with urine or stools a preliminary removal of such material is essential for accurate results.

*After submitting this paper for publication we received the August number of the Biochemische Zeitschrift in which Balint (Bálint, M., *Biochem. Z.*, 1924, cl, 424) describes an iodometric method for the determination of small amounts of sodium in simple solutions of sodium salts.

It occurred to us that the method might be further improved by substituting for the gravimetric determination, an iodometric titration of the antimony in the precipitate or determining the residual antimony in the supernatant fluid and thereby indirectly the sodium. We favored the latter procedure at first because it rendered unnecessary the quantitative separation of the precipitate, thereby avoiding errors due to filtration and washing. In order to titrate the antimony in the supernatant fluid, the protein must be removed by a preliminary ashing. To avoid this step we were compelled to return to the determination of antimony in the precipitate. At the suggestion of Dr. A. T. Shohl we removed the supernatant fluid almost completely after centrifuging and in order to effect a complete separation of serum proteins, added a single washing with 30 per cent alcohol. The method as now carried out is the following.

Method.

2 cc. of serum (or the ash of an equal amount of serum dissolved in 2 cc. of 0.1 N hydrochloric acid and made alkaline with 4 drops of 1.8 N alcohol-washed potassium hydroxide) are placed in a 50 cc. tapering, graduated, Pyrex centrifuge tube which is preferably coated with a thin layer of paraffin.[1] 10 cc. of pyroantimonate reagent are added and then exactly 3 cc. of 95 per cent alcohol redistilled over KOH are added drop by drop while stirring with a rubber-tipped glass rod. The tube is stoppered with a cork and allowed to stand for 30 minutes and then centrifuged for 5 minutes. All but 2 cc. of the supernatant fluid is siphoned off. 10 cc. of 30 per cent alcohol are then added and mixed with the supernatant fluid and the sample is again centrifuged. All of the supernatant fluid possible is then removed by means of a suitable pipette and rubber bulb.

Determination of Antimony in the Precipitate.

5 cc. of 10 N hydrochloric acid (concentrated HCl acid, sp. gr. 1.182) are added to the precipitate and the solution of the precipitate is facilitated by stirring thoroughly with a glass rod. The

[1] Unparaffined tubes of Pyrex or "Exax" glass give equally good results. With unparaffined tubes the precipitate tends to stick to the sides of the tubes.

material is then transferred to a 250 cc. Pyrex beaker, tall form, and transference completed by washing with not more than 10 cc. of distilled water. If the beaker is stirred the material will dissolve completely. At this stage a 15 cc. burette, graduated in 0.02 cc., is filled to the zero mark with 0.1 N sodium thiosulfate. 2.0 cc. of a 20 per cent potassium iodide solution are added to the sample. Free iodine is at once liberated and colors the solution a reddish brown and the specimen is immediately titrated with thiosulfate. The latter should be added very rapidly with constant stirring until the brown color is practically gone. 0.5 cc. of a 1 per cent freshly prepared starch solution is added and the titration continued as before until the sample turns brown when further addition of thiosulfate should be made very slowly with thorough mixing between additions. The end-point is reached when the solution becomes water-clear.

Standardization of the Sodium Thiosulfate.

Approximately 5 gm. of potassium iodide are dissolved in 5 cc. of water. This is poured into a 1 liter Erlenmeyer flask and 10 cc. of 3.33 N hydrochloric acid (concentrated hydrochloric acid 1 part, water 2 parts) are added, followed by exactly 50 cc. of the potassium biiodate solution and 180 cc. of distilled water. The sample is rapidly titrated with 0.1 N sodium thiosulfate until the brown color disappears, when 1 cc. of 1 per cent starch solution is added and the titration continued until the almost black color begins to turn a purple. The addition of sodium thiosulfate is continued with caution drop by drop until the sample becomes water-clear. This is the end-point. The volume of potassium biiodate used (50 cc.) divided by the number of cc. of sodium thiosulfate gives the thiosulfate factor. Thus if 50 cc. of 0.1 N potassium biiodate solution are used and 51 cc. of sodium thiosulfate are required to decolorize the solution, the thiosulfate factor will be 0.98.

Calculations.

Calculation When Antimony Is Determined on the Precipitate.

Since 1 equivalent of iodine is freed by the amount of antimony bound to 0.5 equivalent of Na (see discussion below), each cc. of 0.1 N thiosulfate is equivalent to $\frac{2 \cdot 3}{2}$ = 1.15 mg. of Na. Hence

(No. of cc. of thiosulfate used) × (thiosulfate factor) × 1.15 × $\frac{100}{2}$ = mg. of sodium per 100 cc. of serum or solution when 2 cc. of serum are used. Thus the titration in our analysis was 6.00 cc. of thiosulfate. The thiosulfate factor was 0.96. We have therefore:

$6.00 \times 0.96 \times 0.192 \times 0.5 \times 100 = 331$ mg. sodium per 100 cc. of serum or solution.

Preparation of Reagents.

Potassium Pyroantimonate Reagent.—500 cc. of distilled water are heated to boiling in a Pyrex flask and approximately 10 gm. of potassium pyroantimonate (J. T. Baker) are added. The boiling is continued from 3 to 5 minutes, the flask immediately cooled under running water, and when the contents are cold 15 cc. of 10 per cent KOH (alcohol-washed) are added. The reagent is then filtered through ash-free filter paper into a paraffined bottle. We have found that frequently some of the undissolved potassium pyroantimonate will pass through even the best filter paper. If the reagent is allowed to stand 24 hours after filtering, all the undissolved potassium pyroantimonate will settle to the bottom. The supernatant fluid is then clear and may be used as long as it remains so. The reagent keeps perfectly well at room temperature for at least 1 month. 10 cc. of this reagent will precipitate 11 mg. of sodium. The 10 per cent KOH should be kept in a paraffined bottle.

Before the reagent is used for the first time, it should be tested for the presence of sodium and also the fact ascertained that none of the potassium pyroantimonate is precipitated by the addition of alcohol in the proportion used in the method. This is accomplished by adding to 10 cc. of the reagent, 2 cc. of distilled water and 3 cc. of 95 per cent alcohol.

When ready the reagent should have a reaction of approximately pH 9 and should contain between 63 and 73 mg. of antimony. After 24 hours the reagent is quite permanent, even when kept at room temperature. 95 per cent redistilled alcohol should be used.

Preparation of 0.1 N *Sodium Thiosulfate.*—24.822 gm. of sodium thiosulfate are dissolved in 1 liter of water.

Starch Solution.—1 gm. of soluble starch is suspended in 100 cc. of cold water and this heated until the starch goes completely into solution, giving a water-clear solution. This starch solution does not keep and should, therefore, be made fresh each time.

20 Per Cent Potassium Iodide.—200 gm. of potassium iodide are dissolved in a liter of water. The solution turns slightly yellow on standing.

Potassium Biiodate.—3.2946 gm. of "Kahlbaum, best grade" potassium biiodate are dissolved in 1 liter of water. This is used for the standardization of sodium thiosulfate. The solution keeps in glass at room temperature for at least 1 month.

DISCUSSION.

This method involves several distinct steps: first, the precipitation of the sodium as the pyroantimonate compound; second, the separation of the supernatant fluid from the precipitate by centrifuging, followed by either siphoning or careful aspiration of all the supernatant fluid; third, the solution of the precipitate by means of an excess of *concentrated* hydrochloric acid or the acidification of the supernatant fluid with resolution of the precipitate that forms; fourth, the reduction of antimonic ion with a simultaneous oxidation of the iodide ion of hydriodic acid to free iodine; and lastly, the reduction of free iodine to iodide ion by sodium thiosulfate with the formation of sodium iodide and sodium tetrathionate.

When concentrated hydrochloric acid is added in excess to sodium or potassium pyroantimonate, it forms antimonic acid and potassium or sodium chloride.

$$Na_2H_2Sb_2O_7 + 2HCl + H_2O \rightleftarrows 2NaCl + 2H_3SbO_4 \rightleftarrows 2Na^+ + 2Cl^-$$

$$6H^+ + 2SbO_4^{\equiv} \rightleftarrows [2(OH)_3SbO] \rightleftarrows \underset{-H_2O}{\overset{+H_2O}{\rightleftarrows}} \rightleftarrows 2Sb(OH)_5 \rightleftarrows 2Sb^{+++++} + 10[OH]^-$$

In the presence of an excess of H^+ ions this acid probably conducts itself in the manner similar to arsenic acid; that is, it takes up water and ionizes as a base, yielding antimonic ions and (OH^-) ions. The antimonic ions oxidize iodide ions to free iodine and are in turn reduced.

$$Sb^{+++++} + 2I^- \rightleftarrows Sb^{+++} + I_2$$

We have been unable to find any data as to the quantitative relationships in this oxidation-reduction reaction, but it goes on in acid solution with great rapidity to completion. Qualitatively it must be similar to the one where ferric ion is reduced to ferrous ion in acid solution by iodide ion. Free iodine is again readily reduced by sodium thiosulfate to iodide ion, but in the presence of oxygen and even in its absence the iodide ion shows a very definite tendency to reform free iodine.[2] In acid solutions potassium iodide is, of course, converted into hydriodic acid. The H^+ ions of this acid tend to be reduced to molecular H and iodide ions are in turn oxidized to free iodine.

$$2HI \rightleftarrows 2H^+ + 2I^- \rightleftarrows H_2 + I_2$$

Furthermore iodide ion reacts with the oxygen in the air and water to form free iodine and OH ions according to the equation:

$$4I^- + O_2 + 2HOH \rightleftarrows 2I_2 + 4OH^-$$

Expressing this equation in the form of the mass law we obtain at equilibrium

$$\frac{(I^-)^4 \times O_2}{(I_2)^2 \times (OH)^4} = K, \text{ equilibrium} = 4 \times 10^9$$

If the iodide concentration = 1 molal, the O_2, 20 per cent by volume of the air, the temperature, 25°, and (OH) ion concentration, 1×10^{-14}, the concentration of free iodine at equilibrium will be 10^{22} mols. This indicates the tremendous tendency for the formation of free iodine in acid solution and in the presence of oxygen. The equilibrium constants for the two reactions, namely the conversion of iodide ion to free iodine and of oxygen to OH ions, are known only approximately, but with sufficient accuracy to indicate, as Stieglitz has calculated (3), that in the presence of atmospheric oxygen, iodide ion of a molar solution of hydriodic acid would continue to form iodine until a concentration of 10^{22} mols of free iodine is reached. This indicates clearly that an increase of acidity, of the concentration of iodide or of the oxygen tension, will facilitate the conversion of iodide ion to free iodine, whereas an increase of OH ions even in the presence of only a

[2] This tendency is less marked when the precipitate is titrated.

TABLE I.

Sodium Determinations on a Known Solution of Sodium Chloride.

Sodium per 100 cc. of solution.	
Found.	Present.
mg.	*mg.*
316	313
318	
318	
313	
311	
316	

TABLE II.

Sodium Determinations on 2 Cc. Samples of a Solution Containing Sodium 3.30 Gm. (0.143 Molal), Potassium 0.237 Gm. (0.00608 Molal), Calcium 0.107 Gm. (0.0025 Molal), Phosphorus 0.190 Gm. (0.0061 Molal), Dissolved in 1 Liter of 0.1 N *Hydrochloric Acid.*

Found.	Present.
mg. per 100 cc.	*mg.*
335	330
335	
335	
338	
329	
325	

TABLE III.

Comparative Determinations of Sodium in Ashed and Unashed Serum.

Sodium in unashed serum.	Sodium in ashed serum.
mg. per 100 cc.	*mg. per 100 cc.*
324	327
326	327
328	327
333	338
336	340
324	329

trace of free iodine will prevent any further oxidation of iodide ion to iodine.[3] The reformation of free iodine can be prevented if the solution be made neutral or alkaline, but in such a solution antimony exists as a negatively charged pyroantimonate ion. By using only a small excess of potassium iodide and titrating rapidly to the end-point, the reformation of free iodine during the titration is reduced to a minimum.

Table I gives an idea as to the degree of accuracy of the method when used for the estimation of sodium in solutions of sodium chloride containing sodium in approximately the same concentration as serum.

Table II gives the results of analyses made on solutions containing, besides sodium, the other inorganic components of serum.

Table III is a comparative study of the same method applied to ashed and unashed serum.

CONCLUSIONS.

An iodometric method for the determination of sodium in small amounts of serum, both ashed and unashed, has been described.

This method has a maximum error of plus or minus 2 per cent. The various sources of error have been discussed.

BIBLIOGRAPHY.

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3. Stieglitz, J., The elements of qualitative chemical analysis, New York, 1916, i, 272, 284, 306.

[3] Neutral solutions of potassium iodide acquire a faint yellow only on prolonged standing at room temperature due to the formation of ions of free iodine. This reaction is accelerated when the solution is acidified.